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sensor movement may underlie the destabilization of the fast-inactivated state in NaV1.5. To test this, we expressed NaV1.5 channels in Xenopus oocytes and recorded gating currents using a cut-open voltage clamp with extracellular solution titrated to either pH 7.4 (control) or pH 6.0. At pH 6.0, compared to pH 7.4, the V1/2 of the Q(V) curve was significantly depolarized (from -57.8 ± 4.3 mV to -40.8 ± 5.1 mV). Additionally, the slow time constant of charge recovery was significantly reduced from 16.1 ± 5.0 ms at pH 7.4 to 9.7 ± 4.2 ms at pH 6.0. These data suggest a molecular basis for the increased persistent and window currents previously shown in NaV1.5 channels at reduced extracellular pH. Specifically, protons may electrostatically affect the rate of voltage sensor movement, either by directly binding to extracellular residues (e.g. H880) or indirectly by binding to carboxylates in the pore domain (Kahn et al., J Physiol. 2002, 543). (Supported by an NSERC Discovery Grant to PCR, a CFI Infrastructure grant to PCR and TC, and a CIHR Vanier Scholarship to DKJ.)

1659-Pos Board B429

Direct Evidence of Coupling Between the Selectivity Filter Region and Voltage-Sensor in a Voltage-Dependent Sodium Channel

Deborah Capes[1], Manoel Arcisio-Miranda[2], Brian Jarecki[1], Robert French[3], Baron Chanda[1].
[1]University of Wisconsin - Madison, Madison, WI, USA, [2]Federal University of Sao Paulo, Sao Paulo, Brazil, [3]University of Calgary, Calgary, AB, Canada.

Voltage-gated ion channels are crucial for the generation of action potentials in excitable tissues throughout the human body. In response to membrane depolarization, the movement of the voltage-sensors opens a pore gate on the intracellular side of the protein. Following channel opening, a distinct conformational change in the pore causes a slow loss of channel conductance-a process known as C-type or slow inactivation. However, at a fundamental level, it remains unclear whether these gating transitions in the selectivity filter region are coupled to the movement of voltage-sensors. To investigate this question, we monitored the status of the individual voltage-sensors of a sodium channel upon perturbation of the outer pore conformation by high-affinity toxins and disulfide crosslinking. We introduced a series of mutations in the voltage-sensing domains which resulted in domain-specific gating pore currents and allowed us to monitor the behavior of individual voltage-sensors. We found that in the presence of tetrodotoxin (TTX), the gating pore currents through domain IV (DIV) but not DI, DII, or DIII were reduced. Comparison of the gating charge shows that addition of TTX results in a decrease of total OFF gating charge. Finally, disulphide crossbridge between residues in the DEKA locus of the selectivity filter region also reduces the gating pore currents though DIV. We conclude that the DIV voltage-sensor is specifically coupled to the conformation of the selectivity filter region of the sodium channel. These findings provide direct evidence for the existence of a distinct mode of electromechanical coupling involving the voltage-sensor and gating transitions in the selectivity filter region.

1660-Pos Board B430

A Sodium Channel Voltage Sensor is Targeted by Pyrethroid Insecticides

Eugenio Eduardo Oliveira, Yuzhe Du, Yoshiko Nomura, Gül Satar, Ke Dong.
Michigan State University, East Lasing, MI, USA.

Pyrethroids are well-known insecticides that modify sodium channel gating. It has been long established that pyrethroids slow inactivation and deactivation, which results in prolonged opening of sodium channels. The underlying molecular basis of pyrethroid actions, however, is not well understood. One possible mechanism by which pyrethroids prolong sodium channel opening would be if they promote the outward movement of S4 voltage-sensors and stabilize them in the outward position. In this study, we used the cysteine accessibility method to determine if pyrethroids alter the voltage-dependent movement of the voltage sensor in domain II (IIS4) which has been shown to be critical for sodium channel activation. The outermost arginine in IIS4 was substituted with a cysteine and the accessibility of the cysteine to a hydrophilic cysteine-modifying reagent (MTSET) was measured as the rate of MTSET modification of a cockroach sodium channel expressed in *Xenopus* oocytes. We found that an active isomer (1R, trans) of permethrin, a type I pyerthroid insecticide, increased the rate of MTSET modification, whereas an inactive isomer (1S,trans) of permethrin did not. Our results suggest that permethrin prolongs the opening of sodium channels by trapping IIS4 in the outward position. We are currently examining structurally diverse pyrethroids to further establish this novel mechanism of action of pyrethroids.

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Gating Pore Currents Reveal Sliding Helix Motion of S4 Gating Charges during Activation of NaChBac

Tamer M. Gamal El-Din, Todd Scheuer, William A. Catterall.
University of Washington, Seattle, WA, USA.

Voltage-gated sodium channels are responsible for initiating and propagating action potentials in excitable cells. Transmembrane segment S4 of voltage-gated ion channels resides in a gating pore where it senses the voltage and controls channel gating. Substitution of individual S4 arginines with smaller amino acids allows ionic currents to flow through the mutant gating pore. The voltage-dependence of gating pore currents provides information about the transmembrane position of the substituted cysteine as S4 responds to the membrane potential. Bacterial NaChBac sodium channels are homotetramers. Here we have studied gating pore current in mutant NaChBac channels having one of the four S4 arginines replaced by cysteine. Gating pore current was observed for each mutant channel but with different voltage-dependent properties. Mutating the first (R1C) or second (R2C) arginine to cysteine, resulted in gating pore current at hyperpolarized potentials where the channels are in resting states but not at potentials where the channels are activated. Conversely, mutants R3C and R4C showed gating pore current at potentials where the channels are activated. Our results showing gating pore current for mutants in each gating-charge position with sequential voltage dependence from R1 to R4 demonstrate the stepwise outward movement of the substituted cysteines through the narrow portion of the gating pore that is sealed by the arginine side-chains in the WT channel. This pattern of voltage dependence is consistent with a sliding movement of the S4 helix through the gating pore.

1662-Pos Board B432

Selectivity Filter Region, Tetrodotoxin and Local Anesthetics in the NavAb-based Model of the Nav1.4 Channel

Denis B. Tikhonov[1], **Boris S. Zhorov**[1,2].
[1]Sechenov Institute, St.Petersburg, Russian Federation, [2]McMaster University, Hamilton, ON, Canada.

The x-ray structure of bacterial sodium channel NavAb (Payandeh et al., 2011) provides a new template to model eukaryotic sodium channels, which are targets for many toxins and drugs. Key determinants of tetrodotoxin (TTX) receptor in the outer pore are outer carboxylates in repeats I, II, and IV, three positions downstream from the selectivity-filter residues (DEKA locus). In a Nav1.4 pore-domain model built with S6 and P-loop sequences aligned without indels, the outer carboxylates do not face the pore, whereas repeat III aspartate four positions downstream from the DEKA locus, which according to experiments does not interact with TTX, faces the pore. A point deletion between the DEKA locus and outer carboxylates in each repeat of Nav1.4 improves the sequence similarity of P2 helices and allows to built a NavAb-based model with TTX-Nav1.4 contacts similar to those proposed before. Macro-dipoles of the NavAb P2 helices appear to compensate an excessive negative charge at the EEEE locus, consistent with its sodium selectivity. In the Nav1.4 model, macro-dipoles of P2 helices may compensate an excessive negative charge at the outer carboxylates, while the DEKA locus may be occupied by a sodium ion or a water molecule. The inner pore of the NavAb-based model of Nav1.4 preserves major features of our KcsA-based models, including the access pathway for ligands from membrane through the interface between helices IIIS6, IVS6, and IIIP1. Docking of tetracaine in the Nav1.4 model resulted in the same ligand-channel contacts as proposed by Bruhova et al. (2008). Thus, major aspects of our models of ligand interaction with eukaryotic sodium (and probably calcium) channels do not need major revisions in view of the NavAb structure. Supported by RFBR to DBT and NSERC to BSZ.

1663-Pos Board B433

An In-Silico Approach to Primaquine Binding to Trp756 in the External Vestibule of Sodium Channel Nav 1.4

Eduardo M. Salinas, Angel A. Islas, Thomas F. Scior, Evelyn Martinez-Morales, Lourdes Millan-PerezPeña.
Instituto de Fisiologia-BUAP, Puebla, Mexico.

The aim of our computed study was to examine the possible binding site of primaquine (PQ) using a combined homology protein modeling, automated docking and experimental approach. The target models of wild-type and mutant-types of the voltage-dependent sodium channel in rat skeletal muscle (rNav 1.4) were based on previous work by Tikhonov and Zhorov. Docking was carried out on the P-loop into the structure model of rNav 1.4 channel, in the open state configuration, to identify those amino acidic residues important for primaquine binding. The three-dimensional models of the P-loop segment of wild types and mutant types (W402. W756C, W1239C and W1531A at the outer